Comments of Nathan J. Karch, Ph.D., D.A.B.T. on behalf of The Vinyl Institute on the National Toxicology Program's Classification of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin for the *Ninth Report on Carcinogens*

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Understanding of Mechanism is Insufficient to Justify Proposed Reclassification

Despite its own classification criteria, NTP in its background document, which relies on the IARC monograph, cites an understanding of mechanism as pivotal in upgrading the classification of TCDD. If mechanism is to be used to override the limited nature of the human epidemiologic evidence and NTP's own criteria to justify the elevation of the classification of TCDD to that of a "Known Human Carcinogen," the understanding of mechanism must be clear and complete. It is neither.

Rather than delineating the actual steps in mechanism, IARC and NTP simply cite a role for the Ah receptor and its conservation in mammalian species as the main mechanistic information central to extrapolating animal findings to humans. IARC states:

> 2,3,7,8-TCDD is a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor; this receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals... (p. 343).

However, principal reliance on the role of the Ah receptor as a basis for a mechanism common to humans and animals is overly simplistic and a misrepresentation of the state of the science. The array of recent mechanistic research highlights the inadequacy of Ah receptor binding as a mechanism of action for TCDD-induced carcinogenesis in animals, much less in humans.

Understanding of Mechanism of Carcinogenesis is Limited

Binding to the Ah receptor may be the initial step in the mechanism of tumorigenesis. However, while theories abound, the link between tumor production and Ah receptor binding with nuclear translocation of the ligand-receptor complex is not well understood and may not be direct. Modulation of gene expression subsequent to binding of the ligand-receptor complex is cited as the next step in mechanism, and numerous candidates for the critical genes and gene products have been proposed and are being researched, included growth factor receptors, cell cycle regulators, and others (reviewed in Safe 1995). However, none of these endpoints is so well understood that we can say that we understand how rat liver tumors form, much less how tumors in other tissues or species might occur. Differences from tissue to tissue and from species to species in presence or absence of particular genes, and differences in the kinetics of their expression or interactions with other factors, will greatly affect the likelihood of tumor production.

Even if the Ah receptor is involved in tumorigenesis, the full sequence of steps from binding to the receptor to tumor production is not known. In contrast to this lack of knowledge, we have a full understanding of the steps from binding of TCDD to the Ah receptor leading to enzyme induction. Induction of cytochrome P450 1A enzyme activity burden at all doses tested. In particular, tumors in estrogen-responsive tissues were reduced. *In vivo* and *in vitro* results in animals support this conclusion, and results from the Seveso population appear to support this trend in humans (data reviewed in detail in Safe 1995). In the two stage rat liver model, TCDD treatment after administration of an initiator reduces the number and volume of altered hepatic foci at lower doses (see, for example, Pitot 1987). Based on the experimental evidence, TCDD is more likely to act as an **anticarcinogen** than a carcinogen in humans at doses encountered in the environment, and perhaps even at doses in occupational settings. Our understanding of mechanism is clearly too limited to override the limited human epidemiologic evidence and upgrade the classification of TCDD.

Interspecies Differences are Substantial

Great differences exist between animals and humans in how the body handles TCDD. This is reflected in differences in half-life, distribution in various organs, and in the striking qualitative and quantitative differences in toxicity among animal species and between humans and animals. Toxicity is believed to be the result of changes in gene expression for numerous proteins and factors. The genetics and kinetics of expression of these factors are likely to vary among tissues and among species; thus differences in toxicity should not be unexpected. These differences in toxic responses highlight the peril in over-interpreting our limited understanding of the consequences of binding to the Ah receptor and the other factors and events that may be necessary to elicit toxicity in general, and carcinogenicity in particular, in animals or in humans.

Conclusion

Our lack of understanding of the details of mechanism and the validity of extrapolation between tissues and species is obvious. Given

- the lack of detailed understanding regarding mechanisms of tumorigenesis even in rat liver tissue;

- the likelihood that numerous gene products and other factors not yet understood play critical roles in tumorigenesis in the rat; and

- the active research occurring into mechanisms that may be independent of the Ah receptor,

it is a gross oversimplification to state that we understand the mechanism of carcinogenicity.

Our current level of understanding of the mechanism for tumor induction does not justify overriding the limited nature of the human epidemiologic evidence. The data for TCDD clearly place it in the category "Reasonably Anticipated to be a Human Carcinogen." We urge the Board of Scientific Counselors to maintain the current, appropriate classification.

Pitot, H.C. 1987. A method to quantitate the relative initiating and promoting potencies of hepatocarcinogenic agents in their dose-response relationships to altered hepatic foci. Carcinogenisis 8:1491-1499.

Safe, S. 1995. Modulation of gene expression and endocrine response pathways by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. Pharmac. Ther. 67(2):247-281.

Walker, N.J., Miller, B.D., Kohn, M.C., Lucier, G.W., and Tritscher, A.M. 1998. Differences in kinetics of induction and reversibility of TCDD-induced changes in cell proliferation and CYP1A1 expression in female Sprague-Dawley rat liver. Carcinogenesis 19(8):1427-1435.

Table 1: Confounding Exposures for Some TCDD-Exposed Cohorts

Population	Known Confounding Exposures	References
NIOSH (U.S.)	Over 150 chemical exposures at Plant 10, which accounted for 67 percent of the excess lung cancers, and in which lung cancer was reported to be elevated even in workers not exposed to TCDD Smoking	Delzell et al. 1994 Fingerhut et al. 1991
Boehringer-Ingelheim (Germany)	Benzene Dimethyl sulfate Lindane Hexachlorocyclohexane Smoking	Flesch-Janys et al. 1998
Netherlands	DDT Sodium arsenite Hexachlorocyclohexane Lindane Toxaphene Solvents Amine compounds Mono- and Tri-chlorobenzene More than 60 other industrial chemicals and pesticides Smoking	Bueno de Mesquito et al. 1993